BIOLOGICAL PROPERTIES OF THE NIIEG TULAREMIA VACCINE STRAINS

TRANSLATION NO. 1368

June 1965

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

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[Following is the translation of an article by L. V. Sirotyuk, L. A. Tarasevich State Control Institute for Medical Biological Preparations, appearing in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), #10, 1964, pages 116-120. It was submitted on 7 Aug 1963. Translation performed by Sp/7 Charles T. Ostertag Jr.]

With the aim of specific prophylaxis of tularemia in the USSR, the live Gayskiy--Elbert tularemia vaccine has been used successfully for around 20 years. Initially for the production of this vaccine the Gayskiy No. 15 and the Ondatra-4 vaccine strains were used, however, in the second of these soon after the immunogenicity was lowered so much that it had to be excluded from production. After several years certain changes were exposed in the properties of strain No. 15.

It was established by the experimental investigations of Motornaya (1953) and Yemelyanova (1957) that it was possible to restore the lowered immunogenicity of the vaccine strains by means of passaging them through the organism of animals which are susceptible to tularemia -- white mice or guinea pigs. Along with this, at the Institute of Epidemiology and Hygiene (NIIEG) the new tularemia vaccine strains No. 10, 33 and 53 were isolated, studied and put into use. These were used for a number of years in the production of live NIIEG dry tularemia vaccine together with Gayskiy strain No. 15 (Faybich and Tamarina, 1946).

In 1950, with the aim of increasing and stabilizing their immunogenicity, the NIIEG vaccine strains were passaged 10 times through the testicles of guinea pigs (Motornaya, 1953). Standard samples of these strains, prepared in 1952 in the form of dry lyophilic cultures in a saccharose-gelatin medium, were stored without reseeding for 10 years at 2--10°. The mission of our work was the study of the biological properties of the stated standard cultures of NIIEG vaccine tularemia strains with the aim of clearing up the possibility of using them in the future for the production of live vaccine.

Four strains were taken for study: NIIEG strain No. 10, obtained in 1944 by means of selecting weakly virulent reference cultures (Faybich and Tamarina, 1947), NIIEG strain No. 33, obtained by Faybich,

Saltykov and Tamarina in 1945 also by means of selecting reference strains with a reduced virulence, NIIEG strain No. 53, isolated by Saltykov in 1947 by means of selecting colonies from a culture of virulent strain No. 3, and Gayskiy strain No. 15 restored -- the standard culture of 1962, checked by the commission in 1962 and used at the present time in the production of live tularemia vaccine. As tests for a comparison of the biological properties of the vaccine strains we studied the cultural-morphological properties, agglutinability, residual virulence for white mice, inoculation suitability on guinea pigs, immunogenicity in tests on mice and guinea pigs, and harmlessness for guinea pigs. The dry standard cultures were sown on vitelline medium. In the test we used 48-hour cultures of the second generation on this medium.

All the strains were typical based on the morphology of the cells, tinctorial properties, and nature of growth on nutrient media (typical growth on vitelline medium and fish-yeast blood agar with cystine and glucose, and the absence of growth on ordinary media). On the fish-yeast blood agar with cystine and glucose, the colonies of "immunogenic" microbes in strain No. 10 comprised 98.5%, No. 53 -- 99.9%, No. 33 -- 99%, and in the standard Gayskiy strain No. 15 -- 99.9%. All the strains were agglutinated up to titration by the tularemia diagnostic serum of the Odessa Institute of Epidemiology and Microbiology (titer 1:2,000) and yielded a coarse cottony agglutinate, with the exception of strain No. 33 which yielded a fine cottony agglutinate.

Following the administration to white mice (weighing 18--20 g) of 100, 1,000, 10,000, 100,000 and 1,000,000 microbial cells, the "residual virulence", that is, the death of mice in the vaccinal period, summarily for all doses comprised: In strain No. 10 -- 91%, in No. 33 -- 11%, in No. 53 -- 59%, and in No. 15 -- 30%. Following the subsequent infection of mice which had survived the process of immunization with 1,000 Dcl of virulent strain No. 503 (Dcl = 1 microbial cell based on the standard of the State Control Institute) all the mice remained alive for the period of 15 days of observation (table 1).

The immunogenicity of the strains was also checked on guinea pigs weighing 380-400 g. Five guinea pigs each were vaccinated with a culture of each strain in doses of 1,000, 10,000 and 100,000 microbes. After 27 days the animals were infected with 1,000 Dcl of virulent strain No. 503. Following vaccination with a culture of strain No. 33, two out of the five pigs which had received 10,000 microbial cells died, and four out of the five which were inoculated with 1,000 microbial cells. All the guinea pigs which were vaccinated with cultures of strains No. 10, 53 and 15 remained alive during the course of 30 days observation after infection (table 2).

For determining the inoculation suitability of the strains we

obtained a suspension of microbes in a physiological solution with an optical density corresponding to 1 billion microbial cells, and then prepared 10-time dilutions of it. Guinea pigs weighing 400-450 g were inoculated cutaneously (by the method of scarification) from dilutions of the suspension of 10^{-1}, 10^{-2} and 10^{-3} (two guinea pigs for each dilution). The inoculation reaction in all the animals was expressed sharply (infiltrate and hyperemia around the scarification with a dimension of 0.5 -- 1.2 cm).

The harmlessness of the strains was studied on guinea pigs weighing 350-400 g by means of the subcutaneous administration of a suspension of microbes in a physiological solution with an optical density corresponding to 1 and 3 billion microbial cells (based on the standard of the State Control Institute). Three pigs were taken for each dose. All the tested strains turned out to be harmless.

If our data on the study of the NIIEG vaccine strains is compared with the data of Motornaya (1953), then it becomes apparent that in the process of a ten year storage in a dried condition, these strains hardly changed: The degree of residual virulence for white mice and guinea pigs did not increase and the cultural-morphological properties and agglutinability were preserved completely.

To sum up the study of the NIIEG vaccine tularemia strains No. 10, 33 and 53 in a comparison with the standard Gayskiy strain No. 15, it was established that strains No. 10 and 53 with an increased residual virulence for white mice turned out to be harmless for guinea pigs weighing 350-400 g in a massive dose of 3 billion microbial cells. Besides this, in due time the vaccine prepared from these strains, during mass use on humans, answered the requirements for reactogenicity (Zlatkovskiy et al, 1947; Olsufyev, 1953). Based on all the remaining indices, these strains completely satisfied the requirements set forth for vaccine strains. Consequently they can be recommended as reserves for the production of live tularemia vaccine.

Strain No. 33, based on the data of Motornaya, had a lower immunogenicity than the remaining vaccine strains, and in the agglutination reaction formed a fine cottony agglutinate. According to our data it also yielded to strains No. 10, 53 and 15 in respect to immunogenicity and agglutinability. In connection with this, we consider it unsuitable for industrial use.

Strains No. 10, 33 and 53 preserved all the properties after a 10-year storage in a dried state. This may be explained both by the high quality of desiccation as well as the stability of the strains

themselves, which they apparently acquire during the process of intratesticular passaging through the organism of guinea pigs. Such stability merits attention and requires further study in the process of storing these strains on nutrient media and in passaging through the organism of animals.

Conclusions

1. The NIIEG vaccine tularemia strains No. 10, 33 and 53 practically preserved all their biological characteristics in the process of a 10-year storage in a dried state (in saccharose-gelatin medium).

2. The NIIEG vaccine strains No. 10 and 53 are harmless for guinea pigs and highly immunogenic in tests on white mice and guinea pigs, but possess an increased residual virulence for white mice in comparison with the standard Gayskiy No. 15 strain.

3. The NIIEG vaccine strains No. 10 and 53, based both on their biological properties disclosed at the present time as well as in a test on their wide application in the past, may be recommended as reserves for the production of live tularemia vaccine.

4. The NIIEG vaccine strain No. 33, based on agglutinability and immunogenic properties, is inferior to the other strains and cannot be recommended for the production of live vaccine.

Literature

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Table 1

Residual virulence and immunogenicity for white mice of the NIIEG vaccine strains

No. of strain	Vaccination dose (number of microbial cells)	Residual virulence		Immunogenicity against 1,000 Dcl of a virulent strain	
		Absolute number of dead (numerator) from total number (denominator)	% summarily for all doses	Absolute number surviving (numerator) from total number (denominator)	%
10	100 1,000 10,000 100,000 1,000,000	15/20 18/20 19/20 19/20 20/20	91	9/9	100
33	100 1,000 10,000 100,000 1,000,000	2/20 2/20 2/20 0/20 5/20	11	89/89	110
53	100 1,000 10,000 100,000 1,000,000	5/20 12/20 9/20 13/20 20/20	59	41/41	100
15 Gayskiy	100 1,000 10,000 100,000 1,000,000	2/20 7/20 4/20 5/20 12/20	30	80/80	100

Table 2

Immunogenicity of vaccine strains for guinea pigs

No. of strain	Vaccination dose (number of microbial cells)	Number of survivers (numerator) out of total number infected (denomenator) with 1,000 Dcl	
		1952 (date from Motornaya)	After 10 years of storage 1962
10	1,000 10,000 1,000,000	5/5 10/10 5/5	5/5 5/5 5/5
33	1,000 10,000 1,000,000	1/5 10/10 5/5	1/5 3/5 5/5
53	1,000 10,000 1,000,000	5/5 10/10 5/5	5/5 5/5 5/5
15 Gayskiy (standard 1962)	1,000 10,000 1,000,000		5/5 5/5 5/5